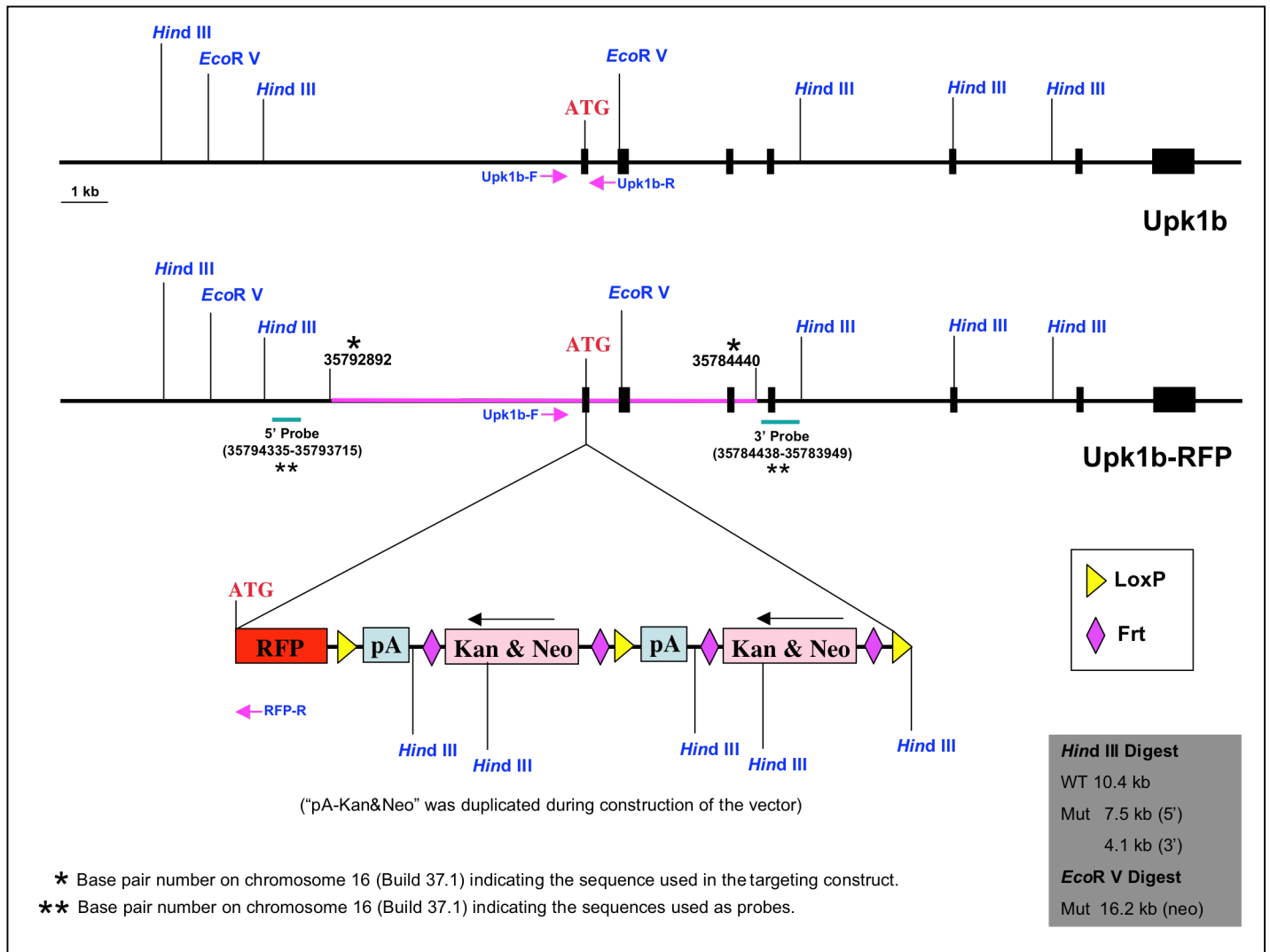


Upk1b

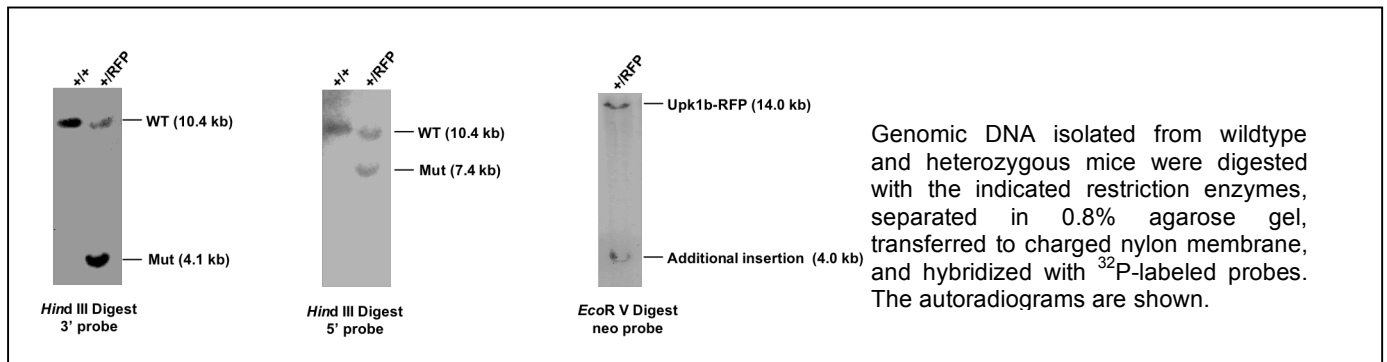
A. Rationale

Four major uroplakin (Upk) proteins (Upk1a, Upk1b, UpkII and UpkIII) are expressed in the urothelium of the bladder and ureter. Upk1a/UPII and Upk1b/UPIII pairs form and these integral membrane proteins are key to the formation of a functional permeability barrier by the bladder by maintaining the physical integrity of the urothelium. These proteins are very abundant in the umbrella cells of the urothelium of the bladder beginning at about E15. We chose to use the Upk1b gene to express RFP to mark these cells because no other marker was available with this specificity. The Upk1b-RFP line generated here demonstrates the same specificity and timing in RFP expression as the endogenous gene.

B. Targeting Strategy



C. Southern Blot Analysis of the Targeted Allele in Mice



Southern blot analysis with neo probe indicates this strain contains an additional insertion and the insertion is linked to the Upk1b^{RFP} as it persists after several rounds of breeding. Because of the size (~4.0 kb) is much smaller than the targeting construct, this additional insertion should not contain RFP sequences. Moreover, the actual size of EcoR V fragment of Upk1b^{RFP} detected by neo probe is smaller than predicted by genomic sequence. This discrepancy is likely because that Upk1b^{RFP} was derived from 129, not C57BL/6.

D. PCR Genotyping

a. Primers

Upk1b-F: 5' agtcctgtgatggctgaccg 3'
 Upk1b-R: 5' aggcactataaaggggaa cg 3'
 RFP-R2: 5' gattcatgcgcttcaaggt 3'

b. Expected Band Sizes

Upk1b-F + Upk1b-R: 425 bp
 Upk1b-F + RFP-R2: 289 bp

E. Relevant Sequences

a. Genomic clone used for targeting construct

```
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Upk1b-F

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Intron/exon junction

Start of RFP

ATGGCCTCCTCCGAGGACGTCATCAAGGAGTTCATGCG
CTTCAAGGTGCGCATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCCGCCCTACGAGGG

RFP-R2

CACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCTGCCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCCAGTA
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End of RFP

gcagcccaattccgatcatattcaataacccttaat

LoxP

Start of

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bGH polyA

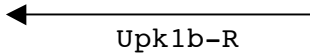
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End of bGH polyA

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Exon/intron junction

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d. 3' probe

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